A sideways look at sparks, quarks, puffs and blips

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An important advance in our understanding of excitation–contraction coupling was provided by the demonstration of calcium sparks attributed to the release of calcium ions from single sarcoplasmic reticulum (SR) release sites (Cheng, Lederer & Cannell, 1993). Subsequent work has shown that these sparks summate to produce the normal systolic Ca^{2+} transient in cardiac muscle (López-López, Shacklock, Balke & Wier, 1994). Sparks have also been found in both skeletal and smooth muscle (Nelson *et al.* 1995; Klein, Cheng, Santana, Jiang, Lederer & Schneider, 1996). In cardiac and skeletal muscle the sparks activate contraction whereas in smooth muscle they appear preferentially to activate Ca^{2+}-activated K^+ currents and thereby promote relaxation (Nelson *et al.* 1995). In smooth muscle the sparks may be the explanation for the spontaneous transient outward currents previously observed (Benham & Bolton, 1986). The sparks do not appear to be restricted to muscle, or indeed to systems using the ryanodine receptor channel (RyR), as closely related phenomena (puffs) have been found in other cells such as the *Xenopus* oocyte where Ca^{2+} release occurs through the $InsP_3$ receptor channel (Yao, Choi & Parker, 1995). These puffs may underlie the spontaneous outward currents seen in cultured neurones (Satin & Adams, 1987).

Cheng *et al.* (1993) originally suggested that the spark might reflect the opening of a single RyR. Two recent papers have, however, suggested that the situation may be more complicated. Lipp & Niggli (1996) addressed the question by using flash photolysis of the 'caged' calcium compound DM-nitrophen. They found that photolysis produced a uniform increase of $[Ca^{2+}]_i$ with no indication of sparks. This was despite the fact that, in the same cell, sparks could clearly be seen during the systolic Ca^{2+} transient. Lipp & Niggli (1996) concluded that flash photolysis could activate a smaller (unresolved) unit of Ca^{2+} release (challengingly christened as quarks) than that provided by the Ca^{2+} transient. Their experiments left unanswered the question of how many quarks and therefore how many RyRs were required to make up each spark. Yao *et al.* (1995) examined the properties of calcium puffs in *Xenopus* oocytes and concluded that up to ten Ca^{2+} release channels could be involved in each puff. Their subsequent work identified calcium 'blips' with amplitudes less than one-fifth of the puffs (Yao *et al.* 1995).

The majority of work on sparks in cardiac cells has examined them by scanning along the long axis of the cell with the confocal microscope. This has the advantage that it is possible to correlate calcium release sites (as shown by sparks) with t-tubules (Shacklock, Wier & Balke, 1995). However, in a paper in this issue, Parker, Zang & Wier (1996) show the results of scanning along the short axis. In the best of physiological traditions, they use a home-made confocal microscope with better spatial resolution than those available commercially. These transverse scans revealed that several sparks could often be detected along one transverse scan. The sparks were spaced less regularly than those along a longitudinal scan. Most interestingly, sparks at adjacent sites were often produced almost simultaneously, suggesting that calcium release from one site may activate an adjacent one. On some occasions, as many as three sites could be resolved and the authors suggest that even more sites may be involved as some sites may not have been resolved, either because they were separated by less than the resolution of the microscope or were out of focus. In this case the basic spark will be smaller than that seen in a conventional line scan. The question then arises as to whether these observations can explain the results of Lipp & Niggli (1996). Specifically, does photolysis of DM-nitrophen activate release from a single site whereas the sparks observed in Lipp & Niggli's experiments were composed of several sites along the transverse axis? This explanation can only work if, during the flash photolysis experiments, for some unknown reason the flash-induced sparks could not propagate and activate all the component sub-sparks at one site.

The work discussed above suggests that sparks and puffs generally consist of smaller subunits (quarks or blips) but does not show how many RyRs make up each of these smaller units. Parker *et al.* (1996) calculated that a single RyR per site open for 5 ms could account for the entire amount of Ca^{2+} released from the SR during an action potential (100 μmol (l accessible cell volume)$^{-1}$). However, as they point out, RyRs are clustered together and it is likely that the Ca^{2+} released from one RyR would activate the others in the cluster. They point out that this apparent paradox could be resolved either if the current through the RyRs *in situ* is less than that observed in artificial bilayers, or if many of the RyRs are 'silent'. We would also point out that from their figures, the Ca^{2+} flux through a single channel would serve to empty the SR, and thus the total release of Ca^{2+} through several open channels would be the same as through one. Therefore, as far as excitation–contraction is concerned, it may not matter enormously how many RyRs open as long as they are clustered together.

Finally, the paper of Parker *et al.* (1996) also shows that the chance of two release sites being coupled decreases as the distance between them increases. At distances corresponding to the separation in the longitudinal direction there is no coupling. This therefore explains why, when examined along the length of the cell, the release sites behave independently. It also provides an explanation for the lack of propagation of locally evoked systolic Ca^{2+} transients (O'Neill, Mill & Eisner, 1990).

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